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mtDNA Control-Region Sequence Variation Suggests Multiple Independent Origins of an "Asian-Specific" 9-bp Deletion in Sub-Saharan Africans

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Summary

The intergenic COII/tRNA^{Lys} 9-bp deletion in human mtDNA, which is found at varying frequencies in Asia, Southeast Asia, Polynesia, and the New World, was also found in 81 of 919 sub-Saharan Africans. Using mtDNA control-region sequence data from a subset of 41 individuals with the deletion, we identified 22 unique mtDNA types associated with the deletion in Africa. A comparison of the unique mtDNA types from sub-Saharan Africans and Asians with the 9-bp deletion revealed that sub-Saharan Africans and Asians have sequence profiles that differ in the locations and frequencies of variant sites. Both phylogenetic and mismatch-distribution analysis suggest that the 9-bp deletion arose independently in sub-Saharan Africa and Asia and that the deletion has arisen more than once in Africa. Within Africa, the deletion was not found among Khoisan peoples and was rare to absent in western and southwestern African populations, but it did occur in Pygmy and Negroid populations from central Africa and in Malawi and southern African Bantu-speakers. The distribution of the 9-bp deletion in Africa suggests that the deletion could have arisen in central Africa and was then introduced to southern Africa via the recent "Bantu expansion."

Introduction

The intergenic COII/tRNA^{Lys} region of human mtDNA usually contains two tandemly arranged copies of a 9-bp sequence (Anderson et al. 1981). Length variation in this region was first inferred by Cann and Wilson (1983), using RFLP, and subsequent sequence analysis demonstrated that the primary length variation was loss of one copy of the 9-bp repeat sequence, CCCCCTCTA (Wrischnik et al. 1987). This deletion has been found at varying frequencies in populations from Asia (Horai et al. 1987; Stoneking and Wilson 1989; Ballinger et al. 1992; Harihara et al. 1992; Passarino et al. 1993; Melton et al. 1995), Polynesia (Hertzberg et al. 1989; Hagelberg and Clegg 1993; Hagelberg et al. 1994; Lum et al. 1994; Redd et al. 1995), and the New World (Schurr et al. 1990; Ward et al. 1991, 1993; Shields et al. 1992, 1993; Torroni et al. 1992, 1994; Horai et al. 1993; Lorenz and Smith 1994) and is commonly referred to as an "Asian-specific" marker (Wrischnik et al., 1987; Hertzberg et al. 1989; Stoneking and Wilson 1989; Shields et al. 1992).

On the basis of the frequencies and the distribution of the 9-bp deletion, it was suggested that the deletion arose once in Asia (Hertzberg et al. 1989). Consequently, this intergenic COII/tRNA^{Lys} 9-bp deletion, in conjunction with both mtDNA control-region sequence variation and RFLP studies, has become one of the more useful mtDNA markers used to trace the genetic trail out of Asia and to reconstruct the biological events associated with the peopling of the Americas and the Pacific (Schurr et al. 1990; Ward et al. 1991, 1993; Shields et al. 1992, 1993; Torroni et al. 1992, 1994; Horai et al. 1993; Lum et al. 1994; Melton et al. 1995; Redd et al. 1995).

However, the deletion was subsequently found at a frequency of 27% in African Pygmies (Vigilant 1990; Chen et al. 1995). This suggests three possibilities for the presence of the deletion in Africans: first, the deletion was introduced to African populations by admixture from recent Indonesian contacts with Africa (Murdock 1959); second, the deletion originated in Africa and subsequently spread to Asia and then to the Pacific and the Americas; and third, the 9-bp deletion originated independently in Africans and Asians.

Phylogenetic analysis of control-region sequence data has indicated that African and Asian mtDNAs with the deletion were not closely related, suggesting separate

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origins for the deletion in Pygmies and in Asians (Vigilant 1990). This conclusion was further supported by Redd et al. (1995), who, using phylogenetic analysis and mismatch distributions, compared control-region sequences from a larger sample of Asians with the deletion to the sequences from Pygmies with the deletion. Chen et al. (1995) also came to this conclusion, by analyzing mtDNA restriction-site variation in the same African Pygmy samples.

These findings raise the question of whether African populations other than Pygmies harbor the 9-bp deletion. We have therefore surveyed 919 sub-Saharan Africans for the 9-bp deletion and have found it at varying frequencies in other sub-Saharan African populations. We further investigated the evolutionary history of the 9-bp deletion in sub-Saharan Africa by sequencing the two hypervariable segments of the mtDNA control region from 41 of 81 individuals with the deletion. These sequences were then compared with (*a*) sequences from 45 Asians with the deletion (Redd et al. 1995) and (*b*) 43 sub-Saharan Africans without the deletion. We used phylogenetic and mismatch-distribution analysis to examine the evolutionary history of the deletion in sub-Saharan Africa and to compare the evolutionary relationship of the African and Asian forms of the deletion. In addition, we examined the concordance of the mtDNA data with the linguistic classification and geographic distributions of sub-Saharan populations with the 9-bp deletion.

Subjects and Methods

Sample

Sub-Saharan African populations have been classified by Hiernaux (1975) into five groups: Bushmen, Pymgy, elongated Africans, western Africans, and Bantu. This classification is based solely on physical anthropological criteria, but other classifications, which incorporate linguistic, cultural, archaeological, and genetic criteria, have also been proposed (see Cavalli-Sforza et al. 1994). The issue of classification is far from being resolved, and genetic studies have revealed that the patterns of genetic variation within Africa (see Cavalli-Sforza et al. 1994) are too complex to resolve by using a single criterion.

For the purpose of this study, we refer to the populations examined herein as Khoisan, Pygmy and Negroid, where Negroid is used broadly to make reference to all non-Khoisan and non-Pygmy populations. To further define the populations tested, we have incorporated their linguistic and geographic locations as well. Sub-Saharan African languages are classified into three families: Khoisan, Niger-Kordofanian, and Nilo-Saharan (Greenberg 1963), and all three are represented in this study (table 1). In addition, sub-Saharan Africa has also been divided geographically, into western, central, eastern, and southern Africa (Parker 1994). In this classification, western Africa is defined as the region that extends between Senegal to the west and Nigeria to the east; eastern Africa refers to the region that includes Ethiopia and Somalia; central Africa, or "equatorial Africa," as it is sometimes called, extends between the Cameroon and Congo Atlantic coastal regions to Kenya and Tanzania along the Indian Ocean; and southern Africa defines the regions between Angola and Mozambique to the southern tip of Africa (fig. 1). The sample of 919 unrelated individuals examined in this study consists of 121 Khoisan from southern Africa, 728 Negroids from various parts of sub-Saharan Africa, and 70 Pygmies from the Central African Republic and Zaire (fig. 1), and these individuals have been grouped by geographic location, into western, central, and southern Africa (table 1).

Western Africa

The western African sample consists of 62 individuals: 14 Yoruba from Nigeria and 48 Gambians (8 Wolof, 9 Jola, 10 Mandinka, 10 Manjago, 4 Serere, and 7 Fula). Western Africans speak mainly Niger Congo languages within the Niger-Kordofanian family of African languages (Cavalli-Sforza et al. 1994).

Central Africa

We have studied 70 Pygmies (20 Mbuti and 33 Efe from Zaire and 17 Biaka from the Central African Republic) and 99 Negroids (22 Lese from Zaire, 17 Hadza from Tanzania, and 60 Luo from Kenya). Efe and Lese samples were provided by R. Boyd; the Luo samples were provided by C. Mason and J. Martinson; and the Biaka and Mbuti samples were from L. Cavalli-Sforza. Whereas the Mbuti Pygmies, the Efe Pygmies, and the Lese speak central Sudanic languages belonging to the Nilo-Saharan language family (Grimes 1992), the Biaka Pygmies speak languages belonging to the Niger Kordofanian family (Cavalli-Sforza et al. 1994). The Hadza speak a Khoisan language, and the Luo speak a Nilotic language from the Nilo-Saharan language family (Grimes 1992).

Southern Africa

This region is inhabited by Khoisan and Bantu-speakers. Populations surveyed from this region are listed by ethnicity, according to the linguistic classification suggested by Greenberg (1963). The Khoisan speakers include 121 individuals (25 !Kung from Botswana, 48 Sekele, and 48 Kwengo originating from southern Angola but obtained in Schmidsdrift in the northern Cape, where they now live) who speak "Bushman" languages and 144 individuals (48 Nama and 96 Dama) who speak a "Hottentot" language (Nama) from Namibia. Whereas the !Kung, Sekele and Nama are genetically similar to one another and have Khoisan characteristics,

Table 1

Geographic and Linguistic Distribution of the 9-bp Deletion in Sub-Saharan African Populations

Region and Ethnic Group	Language Family	No. of Individuals	No. with 9-bp Deletion	Frequency of 9-bp Deletion (%)	No. with Deletion Sequenced
Western Africa:					
Yoruba	Niger-Kordofanian, non-Bantu	14	0	0	0
Gambians	Niger-Kordofanian, non-Bantu	48	0	0	0
Overall, region		62	0	0	0
Central Africa					
Mbuti Pygmies	Nilo-Saharan	20	6	30.0	6
Efe Pygmies	Nilo-Saharan	33	10	30.3	0
Biaka Pygmies	Niger-Kordofanian, central Bantu	17	4	23.5	4
Lese	Nilo-Saharan	22	3	13.6	0
Hadza	Khoisan	17	0	0	0
Luo	Nilo-Saharan	60	1	1.7	0
Overall, region		169	24	14.2	10
Southern Africa:					
!Kung	Khoisan	25	0	0	0
Sekele	Khoisan	48	0	0	0
Nama	Khoisan	48	0	0	0
Kwengo	Khoisan	50	0	0	0
Dama	Khoisan	96	0	0	0
Malawian	Niger-Kordofanian, central Bantu	45	12	26.7	7
Venda	Niger-Kordofanian, central Bantu	41	9	22.0	2
Lemba	Niger-Kordofanian, central Bantu	26	7	26.9	7
Nguni	Niger-Kordofanian, southeastern Bantu	101	13	12.9	9
Tsonga	Niger-Kordofanian, southeastern Bantu	35	2	5.7	2
Sotho/Tswana	Niger-Kordofanian, southeastern Bantu	54	11	20.4	4
Herero	Niger-Kordofanian, southwestern Bantu	69	0	0	0
Himba	Niger-Kordofanian, southwestern Bantu	14	0	0	0
Ambo	Niger-Kordofanian, southwestern Bantu	36	3	8.3	0
Overall, region		688	57	8.3	31
Overall, entire sample		919	81	8.8	41

the Dama and Kwengo are Khoisan-speaking groups who are genetically more closely related to Negroids than to Khoisan people (Nurse et al. 1985, 1987; Soodyall and Jenkins 1992, 1993).

Three major Bantu-speaking groups—central Bantu-speakers, southeastern Bantu-speakers, and southwestern Bantu-speakers—are represented in the sample. The central Bantu-speaking group consists of 45 Malawians. There are 257 southeastern Bantu-speakers in the sample, of whom 41 are Venda, 26 Lemba, 101 Nguni-speakers (40 Zulu, 35 Swazi, 21 Xhosa, and 5 Ndebele), 35 Tsonga-speakers, and 54 Sotho/Tswana speakers (14 southern Sotho, 19 Pedi, and 21 Tswana). The sample of 119 southwestern Bantu-speakers is made up of 69 Herero, 14 Himba, and 36 Ambo individuals, all from Namibia.

mtDNA Analysis

The intergenic COII/tRNA^{Lys} 9-bp region was amplified by the PCR method described by Hertzberg et al. (1989) and was detected on agarose gels as described by Redd et al. (1995). The hypervariable segments of the control region, as well as the intergenic COII/tRNA^{Lys} region, were amplified and sequenced by use of the procedure detailed by Redd et al. (1995).

Computational Analyses

The sequences of 41 individuals with the 9-bp deletion were determined and compared with the published reference sequence (Anderson et al. 1981). Sequence data from 43 Africans (Soodyall 1993; H. Soodyall, M. Stoneking, and T. Jenkins, unpublished data), 4 Europeans, and 7 Asians without the deletion (Vigilant et al. 1991), as well as from 45 Asians or Pacific Islanders with the 9-bp deletion (Redd et al. 1995), were included here for comparisons.

The amount of sequence divergence between each pair of sequences was estimated, after all sites with missing data, insertions, and deletions were excluded, by the *p*-distance method, as implemented by the MEGA computer package (Kumar et al. 1993). Unique lineages were obtained after removal of shared types, and the phyloge-

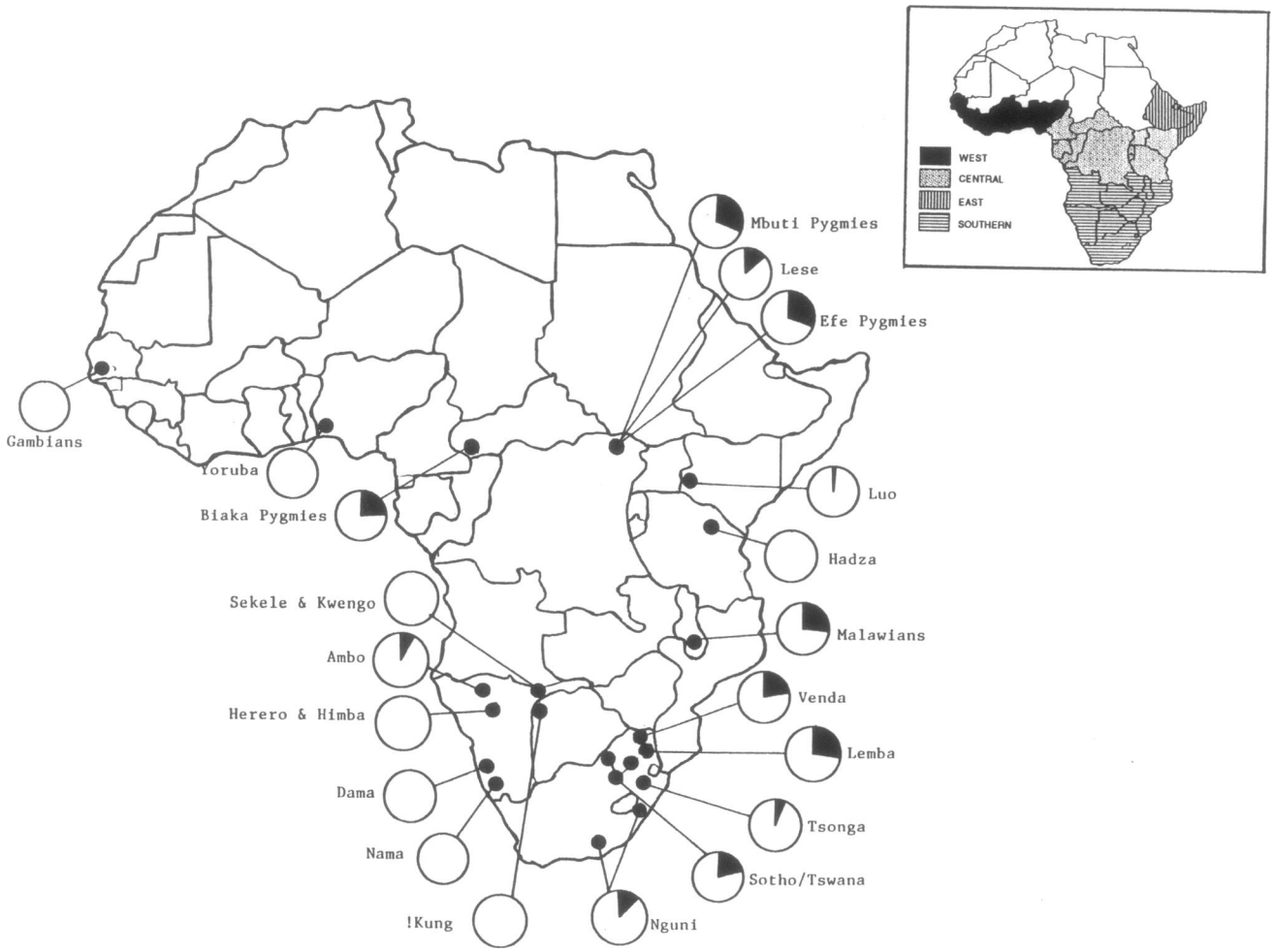

Figure 1 Map showing the four geographic regions within sub-Saharan Africa, and the frequencies of the 9-bp deletion from western, central, and southern African populations.

netic relationships of these sequences were computed by the neighbor-joining (NJ) method (Saitou and Nei 1987). The bootstrap test based on 1,000 replications was applied to estimate the confidence of the branching patterns of the NJ tree (Felsenstein 1975).

We also examined the evolutionary history of the 9-bp deletion in Africans and Asians, using mismatch distributions (Rogers and Harpending 1992; Sherry et al. 1994). The genetic diversity within African populations was calculated from the frequencies of sequence types by use of the method described by Nei (1987, p. 177 [eq. 8.1]). The average rate of mtDNA substitution (λ) for the control region was estimated from the number of nucleotide substitutions between humans and chimpanzees, by use of the substitution model developed by Tamura and Nei (1993) and on the assumption that humans and chimpanzees diverged 4–6 million years ago (Mya). This rate was then used to estimate the time of coalescence (t) for the various clusters associated with the 9-bp deletion, by use of the equation $d = 2\lambda t$ (Nei 1987).

Results

Frequency of the 9-bp Deletion in Africa

The frequencies of the 9-bp deletion in sub-Saharan African populations examined in this study are given in table 1 and are shown in figure 1. The deletion appears to be primarily restricted to central (14.2%) and southern African (8.3%) regions. With the exception of one Luo from Kenya, the deletion is rarely found in east-central Africa and was not found in the western African populations surveyed here (table 1).

Sequence Variation in Africans with the 9-bp Deletion

Sequencing of the intergenic COII/tRNA^{Lys} region from 10 individuals with the 9-bp deletion revealed that the length variation in this region was due to the loss

of one of the 9-bp repeat sequences (data not presented), as is the case with the 9-bp deletion in Asians (Wrischnik et al. 1987). To examine the evolutionary relationship of the deletion in Africans and Asians further, we sequenced ~700 bp of DNA from the two hypervariable segments (HVS1 and HVS2) of the mtDNA control region from 10 individuals from central Africa and 31 individuals from southern Africa with the 9-bp deletion. We then used 685 bp of sequence data to identify the unique mtDNA types, on the basis of pairwise comparisons of the 41 sequences with the 9-bp deletion. We identified 22 unique mtDNA types that define the 9-bp deletion profile in sub-Saharan Africa (table 2).

With respect to the reference sequence (Anderson et al. 1981), we found 35 variant sites, 19 within HVS1 and 16 in HVS2, that describe the 22 unique types found in sub-Saharan Africans (table 2). Although the majority (33 of 35) of substitutions are transitions, transversions occur at position 16188 in HVS1 and at position 132 in HVS2 (table 2). Two types of mutations occur at position 16188, with a G found in 14 mtDNA types and an A found in 4 mtDNA types; the remaining 4 mtDNA types matched the reference sequence in having a C at this position. Since mtDNA types 13 and 14 differ only at position 16188 (table 2), it is likely that first a transversion (either C→G or C→A) occurred at this position (relative to the reference sequence) and that this was followed by a transition (either a G→A or A→G, depending on which type of transversion occurred first). The second transversion, which occurs at position 132, was seen in only one Pygmy individual.

In addition to nucleotide substitutions, insertions and deletions were also found. Insertions were observed in HVS2, between positions 303 and 315, and a deletion of a C at position 98 was found in 8 of 41 individuals with the 9-bp deletion. This deletion is found in some individuals with mtDNA types 2, 3, 6, and 10 (these types were found in only Bantu-speaking groups) (table 2) and is associated only with sequences within cluster 1 of the NJ tree featured in figure 2.

The variant sites that define the African 9-bp profile (types 1–22) were compared with those found in some Asians (types 23–32) with the 9-bp deletion (table 2). Africans and Asians with the 9-bp deletion have sequence profiles that differ with respect to the position and the frequencies of variant sites (table 2), suggesting independent origins of the deletion in Africans and Asians. Also, types 1–18 have several sites in common and usually differ from one another by a few mutational steps, suggesting that they may be derived from the same ancestral type. Types 19 and 20, on the one hand, and 21 and 22, on the other hand, are similar to each other but differ at several positions compared with types 1–18 (table 2). This suggests that types 19 and 20 and types 21 and 22, which are found only in southeastern Bantu-speakers, may represent independent origins of the 9-bp deletion in Africa.

Phylogenetic Analysis

The relationship of the 22 unique mtDNA types found in Africans with the 9-bp deletion was examined on the basis of the patterns of their association in the NJ tree shown in figure 2. This NJ tree resolves the mtDNA types from Africans with the 9-bp deletion into three separate clusters: cluster 1, which consists of mtDNA types 1–18; cluster 2, which consists of mtDNA types 19 and 20; and cluster 3, which consists of types 21 and 22. Although there is good statistical support, by the bootstrap test, for clusters 1 (99%) and 2 (95%) but less support for cluster 3 (69%), the phylogenetic relationship of mtDNA types associated with the 9-bp deletion in Africa (fig. 2) also suggests multiple origins.

To resolve further the question of origins of the 9-bp deletion in Africa, we compared the unique mtDNA types from Africans and Asians with the 9-bp deletion to an additional 54 mtDNA types, obtained from 43 Africans, 4 Europeans, and 7 Asians, all without the deletion (fig. 3). This tree places the unique sequences found in Asians and Africans with the 9-bp deletion into different clusters. Although the tree separates all the sequences found in Asians with the deletion into one cluster, the clusters containing the mtDNA types from Africans with the 9-bp deletion are found in three different branches of the tree, each being separated by sequences from Africans without the 9-bp deletion (fig. 3). Although the bootstrap-support values for these clusters are lower than the values obtained in figure 2 (39% for cluster 1, 91% for cluster 2, and 55% for cluster 3), the patterns of branching of the mtDNA types from Africans with and without the deletion (fig. 3) support the hypothesis of multiple origins of the 9-bp deletion in sub-Saharan Africa.

mtDNA Variation within Populations

We estimated the amount of mtDNA variation within the Pygmy, Malawian, and southern African Bantu-speaking groups, using both the genetic diversity and the mean sequence divergence (table 3), to learn more about the evolutionary history of the 9-bp deletion in sub-Saharan Africa. mtDNA variation within groups is influenced by the number of mtDNA types found within each group and by the amount of sequence divergence between types within these groups. Type sharing was most common among the Pygmy group, with a correspondingly lower value of genetic diversity and sequence diversity, compared with the Malawi and southern African Bantu-speaking groups (table 3).

To assess the effect that types 19–22 had on the estimate of mtDNA variation within the southeastern Bantu-speaking group, we excluded these types and then reestimated the genetic diversity and sequence divergence. Both measures were greatly reduced (table 3); in particular, the mean sequence diversity became much less in southeastern Bantu than in the Malawi or Pygmies. This finding suggests that

Table 2

mtDNA Variation within HVS1 and HVS2 of the Control Region

	mtDNA Control-Region Sequence		
	HVS1	HVS2	
	1111111111111111111111111111111111 6666666666666666666666666666666666 0011111111111222222222222233333 8923444678888111233344466701225 2396078223789278304523716491063	1111111222222333 6789345589900134612 2 4393260295847067351 4	No. of Individuals
Reference sequence[a]	CTGTTCCATACCTATCCACACTACCGATCAT	CATACTCTATCTGATGATGT	
Southern Africans:			
1	.....T.C.TGC...TG.....CT..	T..G...CG...A.CAG...	1 (southeastern Bantu speaker)
2	.....T.C.TGC...TG.....CT..	T..G...CG..CA.CA....	1 (southeastern Bantu speaker)
3	.....T.C.TGC...TG.....CT..	...G...CG..CA.CAG...	2 (1 southeastern Bantu speaker, 1 Malawi)
4	.....T.C.TGC...TG.....CT..	T.....CG..CA.CAG...	1 (southeastern Bantu speaker)
5	.....T.C.TGC...TG.....CT..	.....CG..CA.CAG...	1 (Malawi)
6	.....T.C.TGC...TG.....CT..	T..G...CG..CA.CAG...	12 (3 Malawi, 9 southeastern Bantu speakers)
7	T.....T.C.TGC...TG.....CT..	T..G...CG..CA.CAG...	1 (southeastern Bantu speaker)
8	.....T.C.TGC...TG.....CT..	...G...CG..CA.CA....	1 (southeastern Bantu speaker)
9	.C....T.C.TGC...TG.....CT.C	T..G...CG...A.CAG...	2 (southeastern Bantu speaker)
10	.....T.C.TGC...TG.....CT..	T..G...CG.....CAG...	1 (southeastern Bantu speaker)
11	.CA...T.C.TGC...TG.....CT..	...G...CG.....CA..A.	1 (Pygmy)
12	.C....T.C.TGC...TG.....CT..	...G...CG.....CAG...	3 (Pygmy)
13	.C....T.C.TAC...TG.....CT..	T..G...CG.....CAG...	1 (southeastern Bantu speaker)
14	.C....T.C.TGC...TG.....CT..	T..G...CG.....CAG...	1 (Malawi)
15	.....T.C.TGC...TG.....CT..	.G.G...CGC....CAG...	1 (Malawi)
16	.....T.C.TAC...TG.....CT..	T.C..C.CG.....CAG...	1 (southeastern Bantu speaker)
17	.....T.C.TAC...TG..T.....CT..	T..G.....CAG...	5 (Pygmy)
18	.....T.C.TAC...TG..T.....CT..	T..GG.....CAG...	1 (Pygmy)
19	.....C..T.GT..C.....C...	.G...C...CT....A....	1 (southeastern Bantu speaker)
20	.....C..T.GT..C.....C...	.G...C...C.....A....	1 (southeastern Bantu speaker)
21	.....G..T.CG..TG...C.....C...	.G...C.C.CT....A....	1 (southeastern Bantu speaker)
22	..A.....T.CG..TG...C.....C...	.G...C...CT....A....	1 (southeastern Bantu speaker)
Asians:[b]			
23	.....C.C.....GT.....	.G...C.....G...	26 (type 1)
24	.....C.C.....GT.....	.G...C...C.....G...	2 (type 12)
25	....C.....C.C.....A...G.	.G...CT..C.....G...	2 (type 29)
26	.....C.C.....T.....	.G...C.....G...	8 (type 22)
27	.....T...C..C.C....G.....	.G.....G...	1 (type 32)
28	...C.....C.C.....G....	.G.....C.....G...	1 (type 36)
29	...C.....C.C...T.....	.G.....CA...GCCC	1 (type 35)
30	...C.....C.C.....	.G.....A...G...	1 (type 34)
31	....C.....C.....A.....	.G.....G..G...	2 (type 37)
32	.....C.....A.....	.G.....G..G...	1 (type 38)

[a] Data are from Anderson et al. (1981).
[b] Data are from Redd et al. (1995).

the major form of the deletion, which is associated with mtDNA types 1–18, could have originated in central Africa and that mtDNA types 19 and 20 and types 21 and 22 could have independent origins.

Estimating the Time of Origin of the 9-bp Deletion in Africa

Using the Tamura and Nei model of substitution (Tamura and Nei 1993), we estimated the gamma parameter to be .12, on the basis of the pooled data set of 76 unique mtDNA sequences over 568 sites. This value is similar to the .11 value derived independently from a different data set, used by Tamura and Nei (1993). Incorporating this value of the gamma parameter and using the Tamura and Nei (1993) model of substitution, we estimated the mean pairwise sequence divergence (d) between humans and chimpanzees, based on both transitions and transversions, to be .831 ± .242. By assuming that humans and chimpanzees diverged 4–6 Mya (Vigilant et al. 1991), we estimated that the rate of sub-

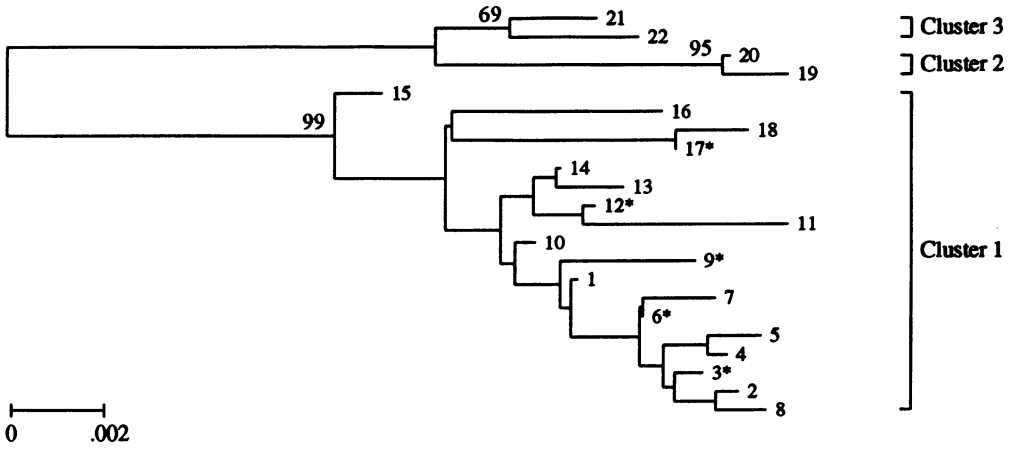

Figure 2 Unrooted NJ tree showing the phylogenetic relationship among the 22 unique mtDNA types found in 41 Africans with the 9-bp deletion. Sequence types found in more than one individual are denoted by an asterisk. The bootstrap support for the three clusters is given as a percentage.

stitution per lineage (with 95% confidence intervals for *d*) is 2.89×10^{-8} (upper limit) and 1.64×10^{-7} (lower limit). The modal rate, calculated under the assumption that humans and chimpanzees diverged 5 Mya, was then estimated to be 8.31×10^{-8}, or 16.6%/million years (95% confidence interval 5.78%–32.88%).

Using these figures for the mtDNA mutation rate, we estimated the time of coalescence, with 95% confidence probability (CP), for the major form of the African 9-bp deletion (cluster 1; fig. 3) to be 72,000 years (95% CP 15,000–173,000 years) (table 4). Since there are only two mtDNA types associated with each of the other two forms of the deletion in Africa (clusters 3 and 4; fig. 3), we did not estimate the time of coalescence of these. However, using the mutation rate of 16.6% and a gamma value of .12 estimated from this data, we estimated the time of coalescence of the Asian form of the deletion (cluster 4; fig. 3) to be 72,000 years (95% CP 12,000–138,000 years) (table 4); a similar value has been estimated by Redd et al. (1995), from a larger sample of Asians with the deletion.

Mismatch and Intermatch Distributions

Using the number of differences between each pair of sequences, we computed the mismatch distributions associated with the 9-bp deletion in Africans (fig. 4*a*). The wave profile reveals two peaks, one with a mean corresponding to 6.5 mutation differences and the other with a mean at 18.5 mutation differences. Removal of mtDNA types 19–22 (sequences contributing to clusters 2 and 3; figs. 2 and 3) eliminated the peak at 18.5 mutation differences (fig. 4*b*), suggesting that the differences between types 1–18 and types 19–22 accounted for peak 2. Mismatch distributions, therefore, are also consistent with multiple independent origins of the deletion in sub-Saharan Africa.

We computed intermatch distributions between Africans and Asians with the 9-bp deletion and derived an interwave distribution with a peak at 22.5 mutation differences (fig. 4*c*), suggesting that the deletion in Africans and that in Asians are due to independent events. Using the 16.6% mutation rate estimated in the present study, in conjunction with the Rogers and Harpending (1992) method, we estimate from this distribution that Africans and Asians with the 9-bp deletion diverged from each other ~114,000 years ago, i.e., before the deletion occurred within each region. Redd et al. (1995) have estimated that the fission between the African and Asian forms of the deletion was ~122,000 years ago; differences between these two measures may be due to (*a*) the additional sequences in this data set that contribute to peak 2 (fig. 4*a*) and/or (*b*) the slightly different mutation rates used in estimating the time of divergence.

Discussion

There are three possible hypotheses to explain the presence of the 9-bp deletion both in Africans and in Asians: (1) a single Asian origin, with migration from Asia to Africa; (2) a single African origin, with migration from Africa to Asia; or (3) independent origins in Africa and Asia. If the first explanation—i.e., that the deletion in Africans is derived from a recent Asian source—is correct, then we would expect to find very little sequence variation between control-region sequences from Asians and Africans with the 9-bp deletion. If the deletion occurred only once in an ancestral population (either African or Asian), as the first two explanations propose, then we would expect to find mtDNA types from Africans and Asians with the 9-bp deletion to be more closely related to one another than either is to mtDNA types found in Africans and Asians without the deletion.

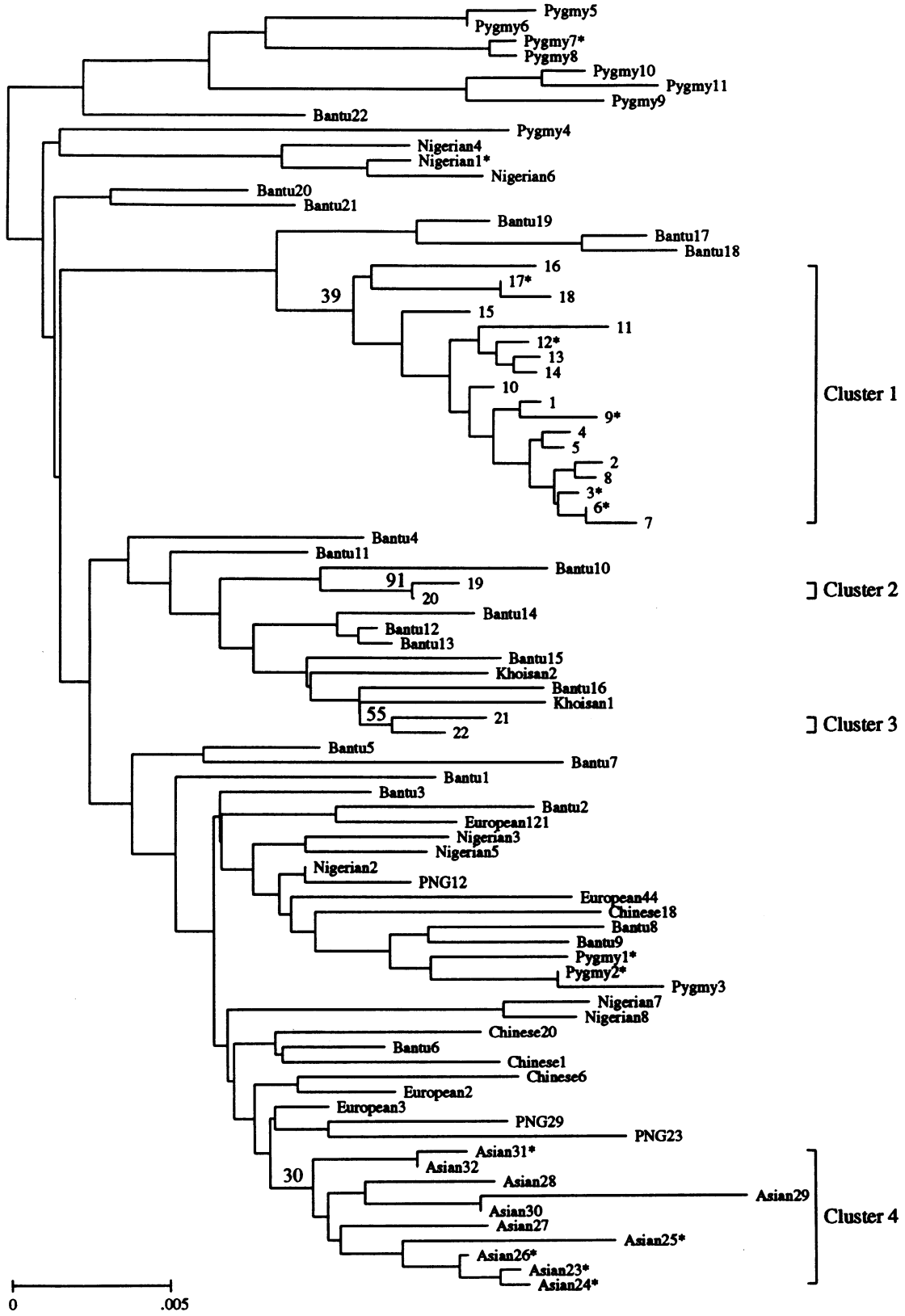

Figure 3 Phylogenetic relationship of mtDNA types from Africans and Asians with the 9-bp deletion, as well as of 43 African (Bantu, Nigerian, Pygmy, and Khoisan), 4 European, and 7 Asian mtDNA sequences without the deletion. The unrooted NJ tree resolves the unique mtDNA sequences from Africans with the deletion into three clusters (1–3) and resolves those from Asians in cluster 4. The bootstrap support for the four clusters containing mtDNA types associated with the 9-bp deletion is given as a percentage.

Table 3

Number of mtDNA Types, Genetic Diversity, and Mean Sequence Diversity in African Populations with the 9-bp Deletion

Population	No. of Individuals	No. of mtDNA Types	Genetic Diversity (*h*)	Mean Sequence Diversity (%)
Pygmy	10	4	.71	.57
Malawi	7	5	.86	.79
Southeastern Bantu:				
Cluster 1 only	20	11	.77	.32
Clusters 2 and 3	4	4	1.00	.79
Overall, population	24	15	.87	1.03
Overall, entire sample	41	22[a]	.90	2.92

[a] Two types were shared by Malawi and southeastern Bantu; see table 2.

If, on the other hand, the 9-bp deletion in Africans and that in Asians are the products of independent events (hypothesis 3), then the sequence profiles of Africans and Asians with the deletion should be distinct; also, mtDNA types in Africans with the 9-bp deletion should be more closely related to mtDNA types in Africans without the deletion, and mtDNA types in Asians with the 9-bp deletion should be more closely related to mtDNAs in Asians without the deletion.

Several lines of evidence from the present study indicate that the sequence profiles of Africans with the 9-bp deletion are unrelated to those found in Asians with the deletion, supporting hypothesis 3. First, the profiles of the variant sites associated with the 9-bp deletion in Africans and Asians are very different (table 2). Many of the nucleotide changes that characterize the African sequence profile are not found in Asians. Also, three substitutions that differ from the reference sequence at positions 16217 (T→C), 16247 (A→G), and 16261 (C→T), which are found at varying frequencies in Asians, Indonesians, and Polynesians with the deletion (Hagelberg and Clegg 1993; Horai et al. 1993; Hagelberg et al. 1994; Lum et al. 1994; Melton et al. 1995; Redd et al. 1995), are not found in Africans with the deletion, implying that the 9-bp deletion in Africa is not related to the 9-bp deletion in Asia.

Second, phylogenetic analysis indicates that the mtDNA types associated with Asians and Africans with the 9-bp deletion are found in different clusters (fig. 3), although bootstrap analysis does not indicate strong statistical support for these clusters. Third, mtDNA types found in Africans and Asians with the deletion are most closely related, respectively, to mtDNA types found in Africans and Asians without the deletion (fig. 3). This suggests that mtDNA types in Africans and Asians with the deletion are independently derived from mtDNA types in Africans and Asians without the deletion, respectively. Also, the 22 mtDNA types found in Africans with the 9-bp deletion are separated into three different clusters, each cluster being separated by mtDNA types found in Africans without the deletion. The association of the 10 mtDNA types from Asians that is shown in figure 3 groups them into a single cluster, a finding similar to that observed by Redd et al. (1995), who examined a larger sample of Asians.

Fourth, the mismatch distribution generated from pairwise comparisons of mtDNA sequences reveals at least two different peaks (fig. 4*a*) associated with the 9-bp deletion in Africans. This suggests multiple independent origins of the deletion in sub-Saharan Africans, thereby favoring hypothesis 3 over the other two hypotheses. In sum, at least two—and perhaps three—separate origins of the 9-bp deletion can be inferred to have occurred in sub-Saharan Africa.

The 9-bp deletion has also been found in an individual of European origin (Torroni et al. 1995). All three forms of the deletion—i.e., that found in Asians, that found in Africans, and that found in Europeans—are associated with different mtDNA types, suggesting that the deletion has occurred multiple times during human evolution

Table 4

Estimation of Coalescence Time for the 9-bp Deletion in Africans and Asians

	$d \pm$ SE	Time of Divergence (95% Confidence Interval)[a] (years)
9-bp Deletion in Africa, cluster 1	.012 ± .005	72,000 (15,000–173,000)
9-bp Deletion in Asia, cluster 4	.012 ± .004	72,000 (12,000–138,000)

[a] Rate is assumed to be 16.6%/million years, on the basis of the present study.

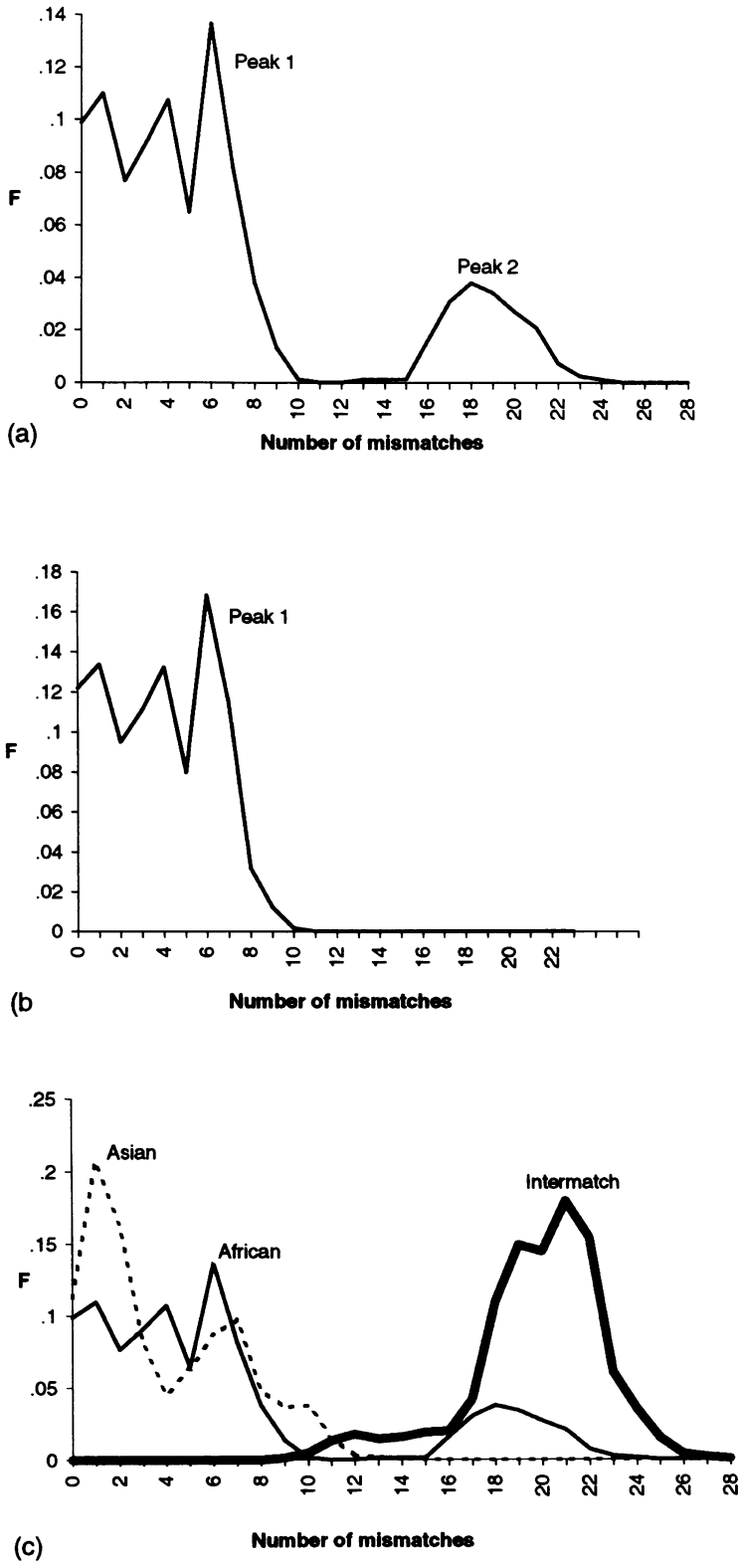

Figure 4 Mismatch distribution showing the genetic structure of Africans with the 9-bp deletion: (*a*) all 41 individuals and (*b*) only those individuals contributing to mtDNA types 1–18. The African and Asian 9-bp deletions were compared by intermatch distributions (*c*); F denotes the relative frequencies of mismatched pairs of sequences.

(Torroni et al. 1995). The findings of the present study supports the suggestion made by Torroni et al. (1995)—that it is no longer correct to refer to the 9-bp deletion as an "Asian-specific" marker—although it is still a useful marker for tracing Asian population history.

In Africa, the distribution of the 9-bp deletion also appears to be informative for tracing population history. In a sample of 919 individuals from various parts of Africa (table 1 and fig. 1) who were surveyed for the 9-bp deletion, we did not find the deletion among western Africans or in the southern African Khoisan populations (table 1). The 9-bp deletion was found at appreciable frequencies in Pygmies (28.6%) from Zaire and the Central African Republic; in the Lese (13.6%), who are Negroids living in close association with the Pygmies in Zaire; in Malawians (26.7%); and in southern African southeastern Bantu-speakers (16.3%). The 9-bp deletion was not found among either 62 individuals from western Africa (present study) or 101 individuals from Senegal (Chen et al. 1995). However, Merriwether et al. (1994) found the deletion in 6 of 189 Nigerians and in 3 of 115 individuals from the Ivory Coast. If we consider this information in conjunction with our data, then the frequency of the deletion in western Africa is 1.9%. Recent gene flow from either central or southern Africans could explain the assimilation of the 9-bp deletion into western Africa.

The 9-bp deletion is also rare in east-central Africa, being found in only one Luo individual from Kenya (table 1). It is possible that the deletion has been introduced recently to this region by gene flow. With the exception of three Ambo individuals from Namibia, the deletion is not found in southwestern Bantu-speakers. Previous studies based on mtDNA RFLP analysis (Soodyall and Jenkins 1993) have shown that the Ambo people, although southwestern Bantu-speaking, have several mtDNA types that are also found in southeastern Bantu-speakers. It therefore has been suggested that some of the female founders of the Ambo descended from southeastern Bantu-speakers (Soodyall and Jenkins 1993), which is supported by serogenetic studies (Nurse et al. 1987). It is likely, therefore, that the deletion in these Ambo individuals is derived from this southeastern Bantu source rather than from an ancestral western Bantu-speaking source. However, other southwestern Bantu-speaking groups need to be studied before it can be claimed that the 9-bp deletion does not occur in these populations.

The source of the deletion in Africans could have been either a Pygmy population or a Negroid population, since the deletion was not found in Khoisan populations. This excludes southern Africa as a likely source for the origin of the 9-bp deletion in Africa, because this region was occupied exclusively by Khoisan peoples before the spread of Bantu-speaking Negroids to the south after the "Bantu expansion" (Clark 1959; Nurse et al. 1985). It has been hypothesized that Bantu languages originated in western Africa in the region between the Cameroon and Nigeria ~3,000 years ago (Guthrie 1962). If the 9-bp deletion originated in the Bantu-language homeland, then there should be a genetic trail out of this region, correlating with migrations that resulted from the Bantu expansion (Guthrie 1962; Greenberg (1963).

One wave of migration, associated with western Bantu culture, is thought to have arisen in the region of the Cameroon grassland ~1600–700 B.C., before spreading to parts of west-central Africa and southwestern Africa (Vansina 1984). If the 9-bp deletion was present in the founding population of western Bantu-speakers, then we would expect to find the deletion in southwestern Africa populations tested (fig. 1). However, the 9-bp deletion is rarely found in southwestern African and western African populations (fig. 1), suggesting that the deletion was not present in the Bantu homeland prior to the Bantu expansion. The presence of the deletion in some western Africans (Merriwether et al. 1994) could be due to recent gene flow or an independent origin of the deletion. A comparison of the control-region sequences from these western Africans to the mtDNA types found in Africans (present study) and Asians (Redd et al. 1995) with the deletion should confirm the origin of the deletion in western Africans.

A more likely source of the 9-bp deletion in sub-Saharan Africa appears to be central Africa, where the deletion is found in both Negroid and Pygmy populations (table 1) and has the highest associated genetic divergence (table 3). Although it must be acknowledged that the classification of Negroid and Pygmy populations used in this study is vague, the 9-bp deletion could have (1) originated in a common ancestor of both Negroid and Pygmy populations in central Africa; (2) originated in Pygmies first and then spread to the Negroids by gene flow, or vice versa; or (3) originated independently in Negroids and Pygmies.

We used the phylogenetic relationship of mtDNA types found in Africans with and without the deletion (fig. 3) to address this question. First, we have found that the major form of the deletion is found in Pygmies from central Africa and in Bantu-speaking Negroids from central and southern Africa. Because their mtDNA types are grouped together in cluster 1 (fig. 3), this excludes hypothesis 3 as a possibility. Second, we find that all three clusters containing mtDNA types from Africans with the 9-bp deletion appear to be more closely related to Bantu-speaking Negroids than to Pygmies without the deletion. Also, the other forms of the deletion in Africa, associated with mtDNA types 19 and 20 (cluster 2) and mtDNA types 21 and 22 (cluster 3), are found only in southern African Bantu-speakers. If the 9-bp deletion was found in an ancestor common to both Ne-

groids and Pygmies (hypothesis 1), then we would expect to find a wider distribution of the deletion in Africa. However, this study shows that the deletion is restricted to central and southern African Negroid and Pygmy populations (fig. 1) and tends to favor hypothesis 2—i.e., that the deletion originated in either the Pygmy or Negroid group and was then assimilated into the other group by gene flow.

If the deletion first occurred in Pygmies and then spread to Negroids, this would explain the presence of the deletion in the various Negroid groups, but then we would also expect to find a closer relationship of mtDNA types from Pygmies with and without the deletion. Since there is a closer association of mtDNA types found in Negroids with and without the deletion (fig. 3), mtDNA data favor the hypothesis that the deletion first occurred in Negroids and then spread to the Pygmies by gene flow.

However, this hypothesis predicts that females from the Negroid population, the transmitters of mtDNA, would contribute their mtDNA to offspring from unions involving Pygmy men and Negroid women; but it is culturally unacceptable for Pygmy males to acquire Negroid wives, whereas Negroid males can readily acquire Pygmy wives (Coon 1965; Cavalli-Sforza et al. 1994). This cultural practice would restrict the assimilation of mtDNA from Negroids into the Pygmies. Although this mode of gene flow between Pygmies and Negroids has been reported from "modern, limited field observations," it does not rule out the possibility that "occasions of more substantial gene flow from Negroids to Pygmies" occurred in the past (Cavalli-Sforza et al. 1994, p. 174). This would then explain the pattern of association of mtDNA types from Negroids and Pygmies that is seen in figure 3. Also, the discrepancy between the mtDNA data and the cultural data may be due to the broad classification of sub-Saharan African populations that we used in this study.

Despite these uncertainties about the origin of the 9-bp deletion in Africa, it is still a useful marker to trace migrations of sub-Saharan populations and to examine the degree of concordance between language, geographic location, and the patterns of mtDNA variation found within Africa. Specifically, we can use the 9-bp deletion to test the routes of migration of Bantu-speakers that have been suggested by linguistic (Guthrie 1962; Greenberg 1963) and archaeological (Phillipson 1985; Huffman 1989) studies. Linguistic and archaeological studies have revealed that one wave of migration took place from west-central Africa to the region of the Great Lakes in eastern Africa and then into southern Africa. Although we have limited population samples along these hypothesized routes of migrations, the spread of the deletion into southern Africa appears to have resulted from this expansion.

None of the Africans with the 9-bp deletion had mtDNA types that were related to Asian mtDNA types with the 9-bp deletion. Therefore, although historical and anthropological studies have traced to Asia several cultural traits found in Africa (Murdock 1959), the mtDNA studies indicate that the people who brought them left no biological evidence of their incursions. This could be due to the fact that very few voyagers made the journey, that there was little or no admixture, or that men only undertook the voyages. Additional genetic studies incorporating markers selected for their Asian specificity in populations along the eastern coast of Africa should be useful in identifying the extent of Asian admixture in sub-Saharan African populations. Y-chromosome haplotypes may be extremely useful in this regard, because they would directly reveal the genetic contribution of males. However, the lack of Asian mtDNA types with the 9-bp deletion in Africa does suggest that the higher mtDNA diversity usually found in African populations (Cann et al. 1987; Merriwether et al. 1991; Vigilant et al. 1991; Soodyall 1993; Chen et al. 1995) is not due to recent admixture from Asia.

Although the Asian and African deletions are clearly the result of different events, the coalescence times are remarkably similar (table 4). This may be just a coincidence (in light of the large standard errors), but it is interesting to speculate that these times (70,000 years or so) are very similar to the times when human populations expanded worldwide, as determined by mismatch-distribution analysis (Sherry et al. 1994). Because there have been multiple origins of the 9-bp deletion in both sub-Saharan Africa (present study) and Asia (Ballinger et al. 1992; Redd et al. 1995), it is likely that the deletion spontaneously occurs regularly during evolution. The usual fate would be loss via drift; but, if the deletion happened to occur when populations were expanding, then it would be less likely to be lost by drift. The persistence and the spread of the primary Asian and African forms may thus reflect "lucky" mutations that occurred at the time of worldwide population expansions.

In conclusion, the 9-bp deletion in sub-Saharan Africans and that in Asians have different origins, and, in fact, several lines of evidence suggest that the deletion arose in Africa more than once. The distribution and the frequencies of the 9-bp deletion in sub-Saharan African populations suggest that its spread in Africa may be due to the recent "Bantu expansion." The 9-bp deletion, in conjunction with control-region sequence data, should thus be a useful mtDNA marker for examining the routes of migration of Bantu-speakers that have been hypothesized on the basis of linguistic and archaeological evidence.

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